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Non-Invasive Serial Blood Collection in Guinea Pigs (*Cavia porcellus*)

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14. ABSTRACT

Blood draws from guinea pigs (*Cavia porcellus*) became a requirement for animals trained in several behavioral tasks pre- and post-exposure to chemical warfare agents (CWAs). Due to the number of animals, the sensitivity of the behavioral assessments and the need for serial blood draws, current methods for blood collection could not be used because they required anesthesia or analgesics, extensive training, a high level of competence, and multiple technicians. The toenail clip method provided an easily trained, easily performed method of blood collection that yielded up to one milliliter of blood, required only topical anesthesia and appeared to cause little pain or stress to the animal.

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Introduction

Current methods for blood collection from guinea pigs (*Cavia porcellus*) include drawing blood from the femoral triangle, anterior vena cava, or saphenous vein and cardiac puncture.[1] These methods require multiple technicians, the use of anesthetics or analgesics, extensive training and technical competence, or they are part of a terminal procedure. The time required to draw blood using these procedures for each animal renders these methods impractical when a large number of animals require multiple blood sampling.

A method was needed that would be easy, require little training, and yield at least 1.0 milliliter of blood but could be easily controlled when less blood was needed. The method should also produce little or no stress on the animals. Such a method was developed from the routine care practice of clipping the nails of the guinea pig.[2] By clipping more nail off than is required for normal care, blood collection is possible.

Materials and Methods

Animals:

Hartley guinea pigs (Crl: (HA)BR), weighing an average of 250 to 300 grams, were provided with water *ad libitum* and housed individually in hanging cages (53.5 L X 28.8 W X 20.5 D cm). All animals were housed in an AAALAC-accredited facility using standards set forth in *The Guide for the Care and Use of Laboratory Animals*.[3] Corncob bedding was provided and two cage changes were completed every week also in accordance with *The Guide for the Care and Use of Laboratory Animals*.[3] Subjects were maintained in a temperature- (20 - 22°C) and humidity-controlled (50% ± 10%) environment with ten complete air changes per hour of 100% conditioned fresh air. Light was provided on an alternating 12-h light/dark cycle with lights on at 0600 h with no twilight. After release from quarantine, all animals were implanted subcutaneously with IPTT™-200 implantable identification/temperature probes (BioMedic Data Systems Inc., Seaford, DE) (Figure 1). Then each animal's ear was marked with a non-toxic marker with a number corresponding to the implantable probe (Figure 2). Guinea pigs were fed 100g of food once per day (Harlan-Teklad Guinea Pig Diet 7006 Madison, WI) until they reached 370 grams. All animals were then placed on 80% of the recommended daily allowance (RDA), or 6g per 100 gram of body weight. Animals were kept on dietary controls for the duration of the study. Animals were handled daily and trained in behavioral tasks of operant, active avoidance, and acoustic startle. Water was available *ad libitum*.

Handling:

Animals were handled daily to acclimate them to the frequent handling they would be exposed to during the course of the observational period and behavioral testing.

Equipment and Supplies:

Participating technical staff wore appropriate personal protective equipment (PPE), which included a lab coat, face mask, gloves (SafeSkin®, Purple Nitrile®, Kimberly-Clark, Roswell,

GA) and safety glasses. Animals were placed on absorbent protective pad during the procedure. Supplies for the procedure included cat claw scissors (Millers Forge, Inc., Plano, TX), general surgical scissors, gauze pads, styptic powder (Kwik-Stop® with Benzocaine, Gimborn Pet Specialties, LLC., Atlanta, GA), pre-labeled blood tubes with EDTA (K_3)(Monoject® Samplette®, Sherwood Medical Company, St Louis, MO), and a permanent marker (Sharpie®, Sanford, Bellwood, IL) to mark all ears. Cat claw scissors and surgical scissors were disinfected prior to use with a solution of Rocal-D (Pfizer, New York, NY) and rinsed with tap water. A sample rocker (Thermolyne, Dubuque, IA) was available for use by all technicians (Figure 3). A laboratory chair was provided for each technician.

Analgesia:

At the completion of blood collection, styptic powder with a local analgesic agent (Kwik-Stop® with Benzocaine, Gimborn Pet Specialties, LLC. Atlanta, GA) was placed directly on the nail. Slight pressure was applied to assure contact with the nail. The animal was returned to the transport cage and observed for continued bleeding and signs of pain (limping), prior to return to home cage.

Technician Training:

All technicians to be involved with blood sampling were trained on this task by an experienced laboratory animal technologist (LATG). All technicians had previous handling experience with guinea pigs.

Blood Collection Procedure:

To pick up the guinea pig, one hand is slowly and gently placed around the thorax of the guinea pig (Figure 4) while care is taken not to squeeze the rib cage. The thumb is placed under a front leg while the first and middle fingers are used to restrain the opposite front leg. The second hand is placed beneath the animal for stability. The second hand slides under the rump of the animal once lifted, to support the guinea pig fully (Figure 5). With gentle but firm restraint the guinea pig is removed from the cage. The animal's face is placed towards the technician's elbow while restraint is maintained on the animal's rump and shoulder (Figure 6).

Once the guinea pig is removed from the cage, the technician carefully sits down in a chair. A blue absorbent pad is placed across the technician's lap to protect against blood drops and to give the animal secure footing during rest periods. The guinea pig is placed along one forearm with feet pointing towards the technician's hand. This placement will cause the guinea pig to cradle between the technician's chest and arm. This allows for a secure, comfortable restraint, as well as a sense of security for the animal. The foot to be bled from is gently extended away from the body (Figure 7). Patience must be used because the guinea pig may resist and try to pull the leg away. Gentle restraint is used to keep the leg in place, without tugging on the leg.

Once the animal is in a secure, comfortable position, blood collection is ready to begin. Either cat claw scissors or general surgical scissors may be used to clip the toenail. With the free hand, the cat claw scissors and blood collection tube are obtained and placed on the blue pad

across the technician's lap. The animal ID number is then checked to make sure it matches the number on the tube (Figure 8). If an error in the number has been made, it can be corrected at this time. The thumb is placed over the top of the foot and the index finger under the foot in a gentle hold to control the foot. This position will keep the toes apart and reduce the risk of accidental nicking of another toenail during the actual clipping.

With the free hand, the cat claw scissors or general surgical scissors are picked up from the lap. The cutting edge is placed around the toe nail at an approximate 45º angle, just below the nail bed (Figures 9 and 10). The actual toe itself is not cut. Only the nail is cut. In one firm, quick movement, the clippers are closed to clip the toenail.

After the toenail has been cut, the scissors or cat claw clippers are put down, and the blood collection tube is picked up with the now free hand. The blood collection tube is placed under the clipped toe and blood is allowed to drip into the tube (Figure 11).

When the appropriate volume of blood is collected, the animal's foot is elevated to temporarily slow the flow of blood while the collection tube is capped. A gauze pad is picked up and pressed to the clipped toenail (Figure 12). Pressure is maintained for 10 to 20 seconds; then the gauze pad is removed. The gauze is removed and the toe is dipped into a small amount of powdered Kwik-Stop® (Figure 13). If the bleeding has not stopped, the hemostatic powder and pressure can be reapplied. If bleeding has stopped the animal can be returned to the transport cage. The animal should be observed for limping and continued bleeding for approximately twenty minutes. Sometimes a residual amount of blood can be seen on the bedding.

Additional monitoring should be done at various intervals following blood collection. Ten minutes after return to home cage one- and four-hour observations after blood collection are suggested to ensure that blood has been properly stopped and the animal appears to be within normal health limits. Animals should be monitored every day during routine observations. Signs of infection or lameness are rare but should be monitored and noted if present.

Discussion

Several factors can affect the rate at which blood flows into the tube and the amount of blood collected. These include exposure to chemical warfare agents, age of the animal, excess in pressure and overt restraint on the foot and failure to cut off enough toenail. Typically animals exposed to higher doses of chemical warfare nerve agents have a slower rate of blood flow. Excited animals will have a faster blood flow rate. Excessive pressure and overt restraint on the foot or the animal can reduce blood flow. Reduction of pressure and restraint can alleviate this problem. If necessary, the same toe nail can be re-clipped but usually within hours of the initial collection: just wiping the toe nail is sufficient to get the blood flowing again. The position of the animal's foot in relation to its heart can also reduce or stop blood flow. Make sure the animal's foot is lower than its heart level. If the foot is higher than the heart, the blood flow to the toes will be restricted. Reposition the foot if necessary to allow gravity to pull the blood to the toes. It is possible to enhance slightly or induce blood flow by gently stroking the top and bottom of the foot with your thumb. Blood clots may form on the end of the toenail. If this happens, use gauze to gently wipe the clot off the toenail to re-open the blood vessels.

The toenail blood collection procedure described here is an effective tool for drawing up to one milliliter of blood. Utilizing this technique, it appears that only momentary pain is experienced, adverse effects of anesthetics to behavioral studies is eliminated and the number of technicians and duration required to collect routine and repeated blood samples is minimized.

Figure 1. BMDS microchip scanner and weight scale.

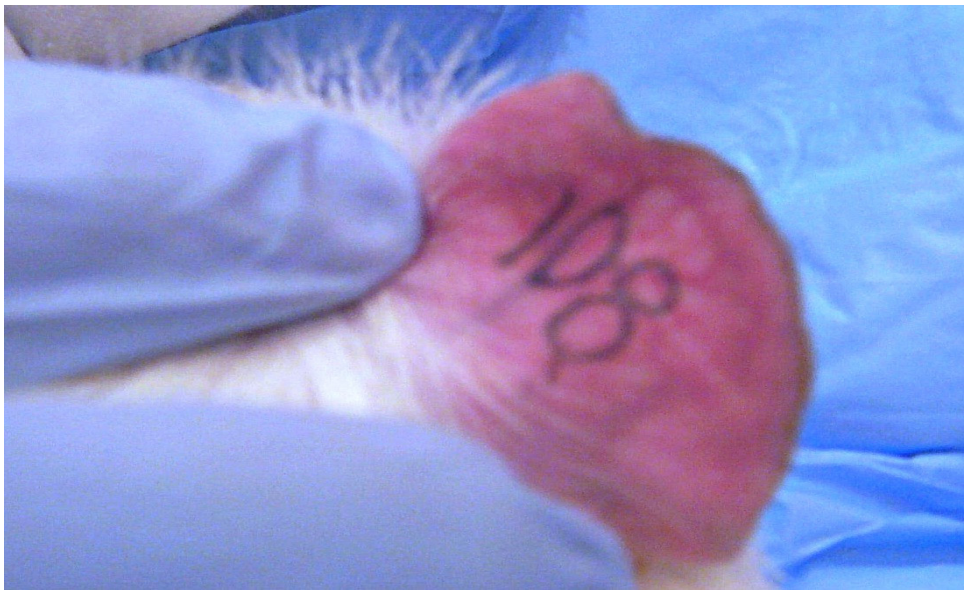

Figure 2. Numbered guinea pig ear.

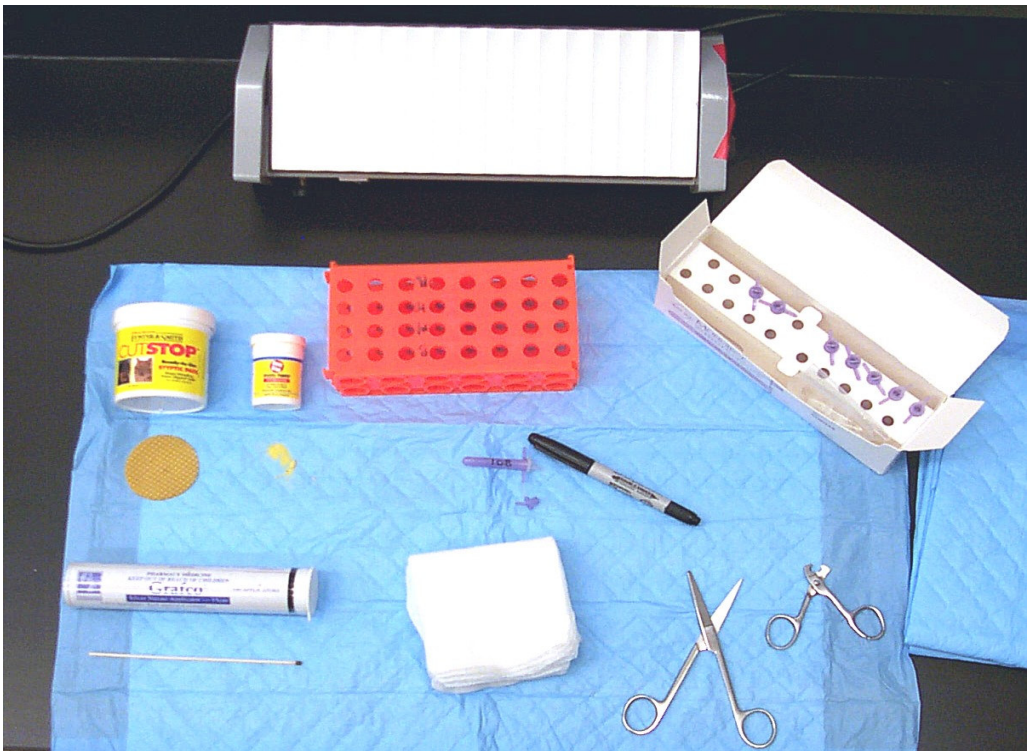

Figure 3. Blood collection supplies.

Figure 4: One hand around the thorax.

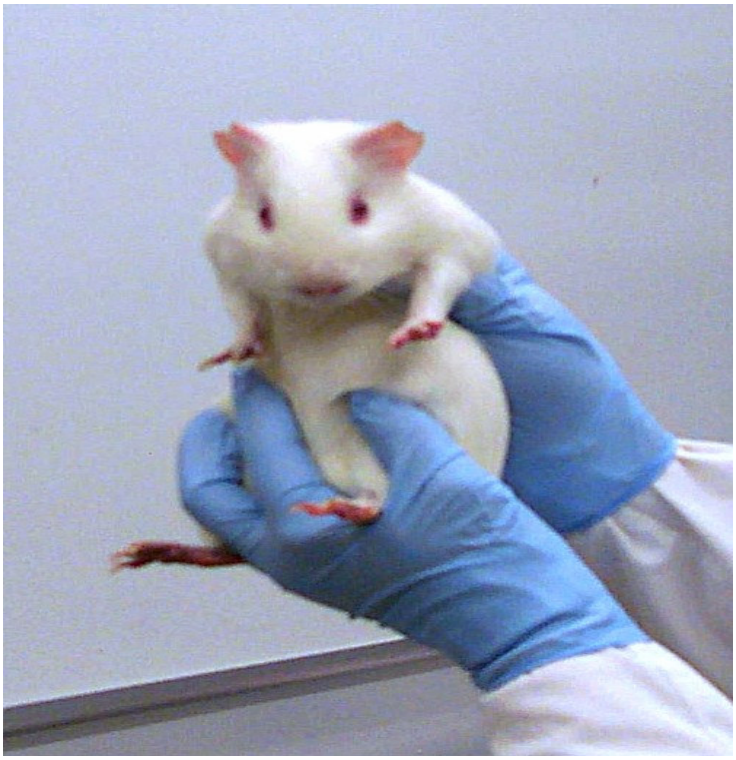

Figure 5. Proper two-handed restraint.

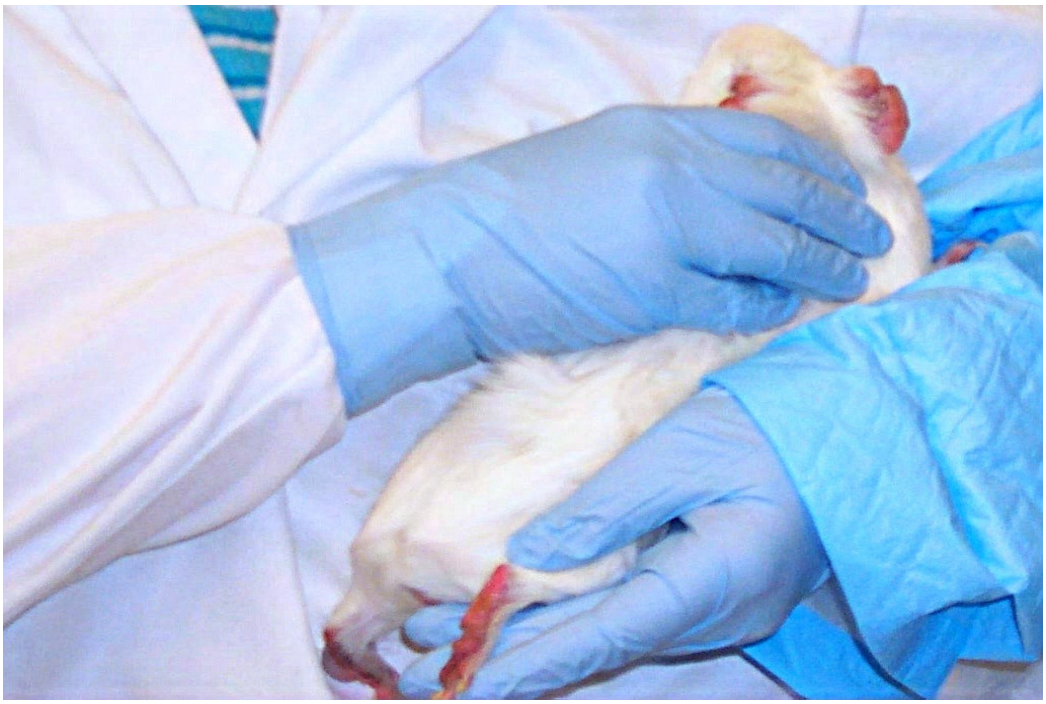

Figure 6. Comfortable restraint for bleeding.

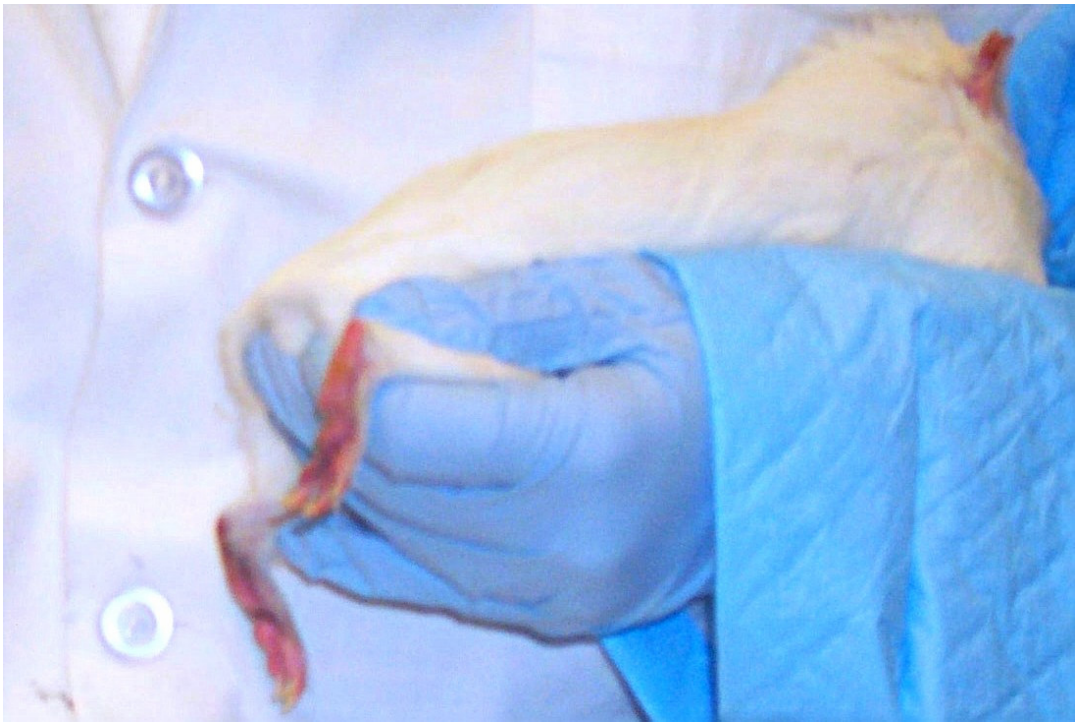

Figure 7. Extend leg and control foot.

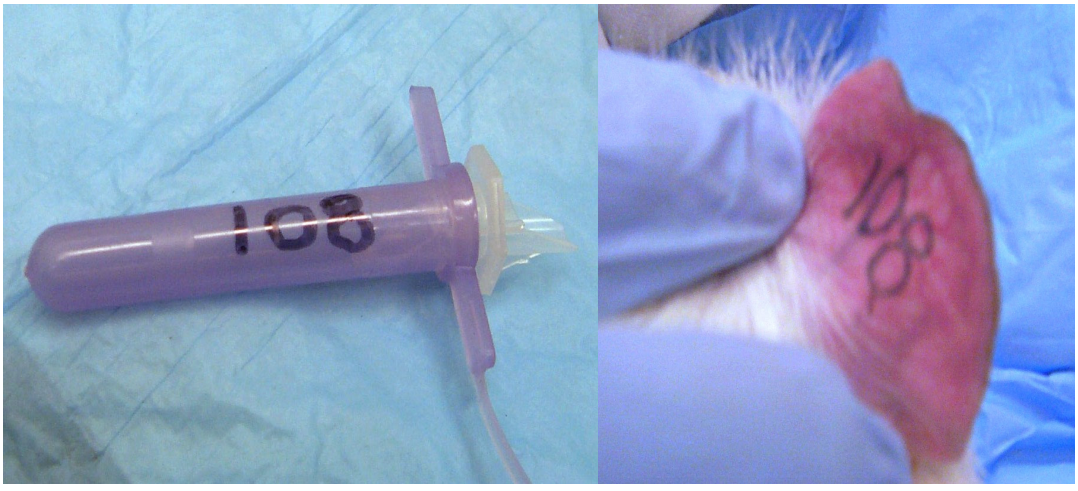

Figure 8. Make sure collection tube and ear match.

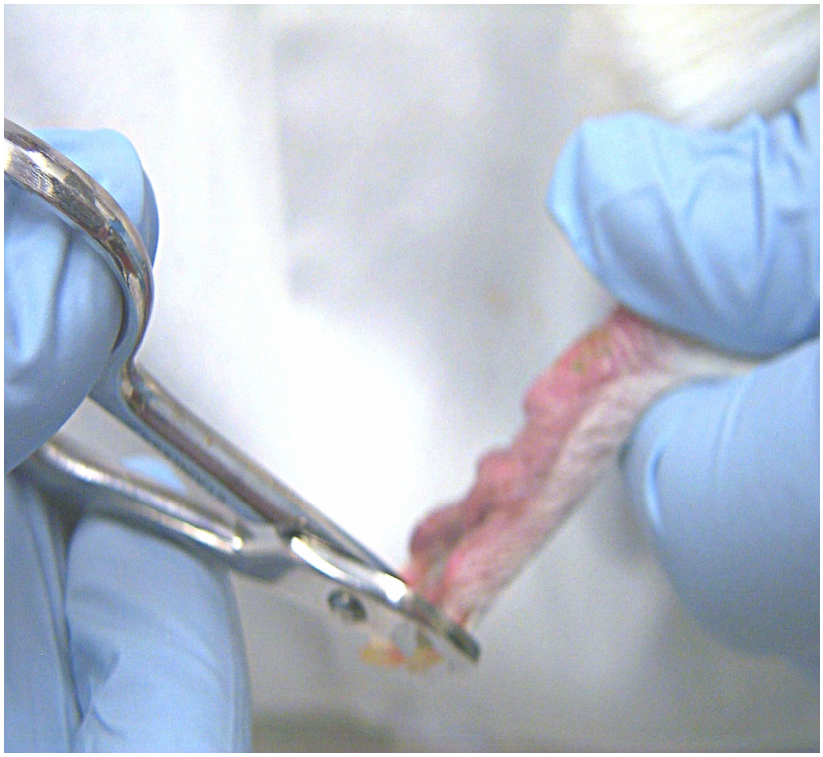

Figure 9. Using cat claw scissors.

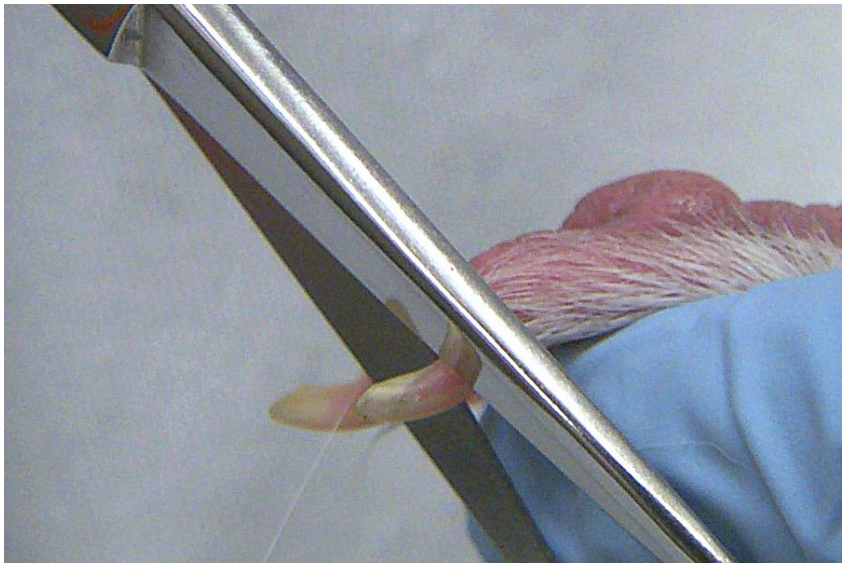

Figure 10. Using surgical scissors .

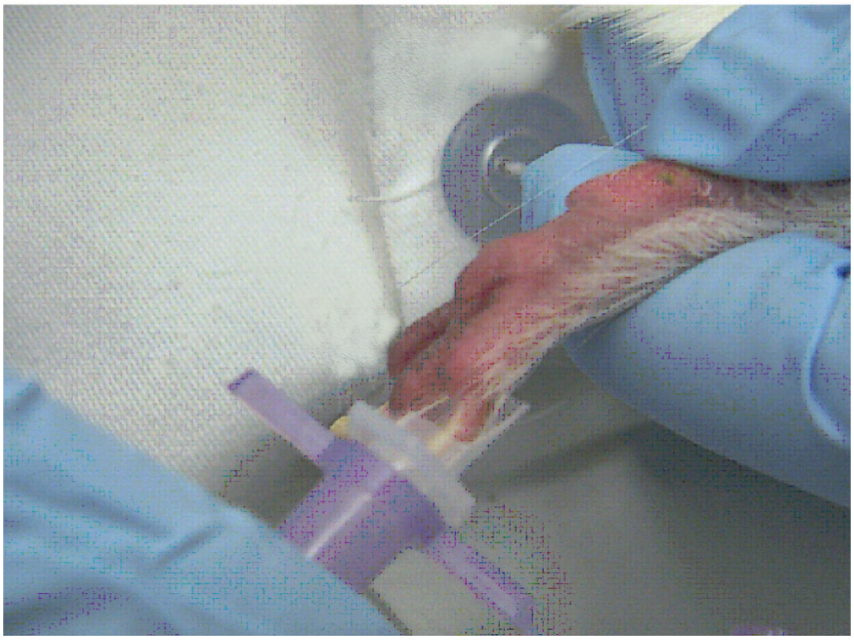

Figure 11. Al low blood to drip into tube.

Figure 12. Wrap gauze around toe and apply direct pressure.

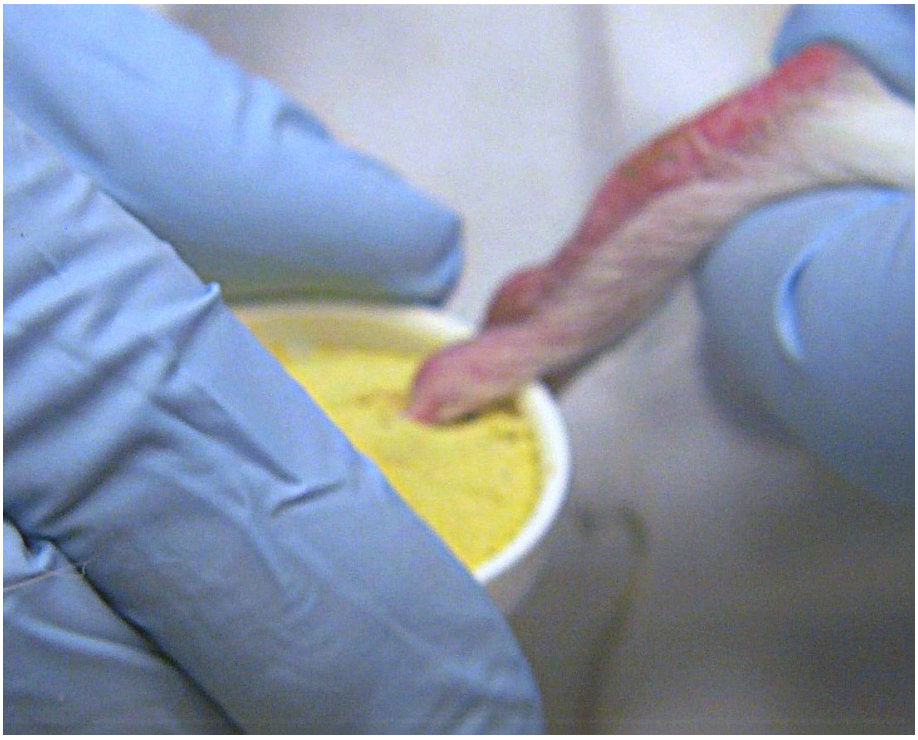

Figure 13. Using Kwik-Stop with benzocaine.

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